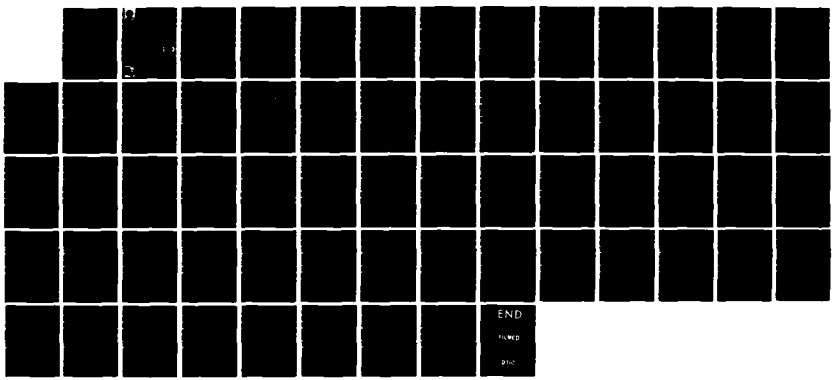
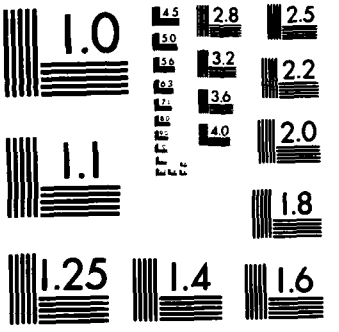
AD-A157 374 LABORATORY EVALUATION OF ADENYLATE ENERGY CHARGE AS A TEST FOR STRESS IN.. (U) ARMY ENGINEER WATERWAYS EXPERIMENT STATION VICKSBURG MS ENVIR.. 1/1

UNCLASSIFIED G E ZARDOGIAN ET AL. FEB 85 WES/TR/D-85-3 F/G 6/1 NL






MICROCOPY RESOLUTION TEST CHART

)ARDS-1963-A

FIELD VERIFICATION PROGRAM
(AQUATIC DISPOSAL)

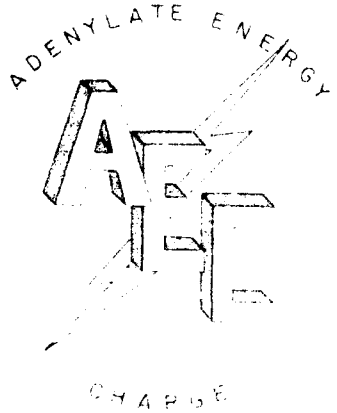
TECHNICAL REPORT D-85-3

# LABORATORY EVALUATION OF ADENYLATE ENERGY CHARGE AS A TEST FOR STRESS IN *MYTILUS EDULIS* AND *NEPHTYS INCISA* TREATED WITH DREDGED MATERIAL

by

Gerald E. Zaroogian, Carol Pesch
Paul Schauer, Diane Black

Environmental Research Laboratory
US Environmental Protection Agency
Narragansett, Rhode Island 02882






February 1985
Final Report





Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

and US Environmental Protection Agency
Washington, DC 20460

Monitored by Environmental Laboratory
US Army Engineer Waterways Experiment Station
PO Box 631, Vicksburg, Mississippi 39180-0631

Destroy this report when no longer needed. Do not return it to the originator.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents

The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products.



The Dissemination of reports includes publications of the Environmental Effects of Dredging Programs:

Dredging Operations Technical Support

Long-Term Effects of Dredging Operations

Verification and Validation of Methodologies for Evaluating Dredged Material Disposal Alternatives

Environmental Effects Monitoring Program

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Laboratory Evaluation of Adenylate Energy Charge as a Test for Stress in Mytilus edulis and Nephtys incisa Treated with Dredged Material"

TO: All Report Recipients

1. This is one in a series of scientific reports documenting the findings of studies conducted under the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives (referred to as the Field Verification Program or FVP). This program is a comprehensive evaluation of environmental effects of dredged material disposal under conditions of upland and aquatic disposal and wetland creation.

2. The FVP originated out of the mutual need of both the Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) to continually improve the technical basis for carrying out their shared regulatory missions. The program is an expansion of studies proposed by EPA to the US Army Engineer Division, New England (NED), in support of its regulatory and dredging missions related to dredged material disposal into Long Island Sound. Discussions among the Corps' Waterways Experiment Station (WES), NED, and the EPA Environmental Research Laboratory (ERLN) in Narragansett, RI, made it clear that a dredging project at Black Rock Harbor in Bridgeport, CT, presented a unique opportunity for simultaneous evaluation of aquatic disposal, upland disposal, and wetland creation using the same dredged material. Evaluations were to be based on technology existing within the two agencies or developed during the six-year life of the program.

3. The program is generic in nature and will provide techniques and interpretive approaches applicable to evaluation of many dredging and disposal operations. Consequently, while the studies will provide detailed site-specific information on disposal of material dredged from Black Rock Harbor, they will also have great national significance for the Corps and EPA.

4. The FVP is designed to meet both Agencies' needs to document the effects of disposal under various conditions, provide verification of the predictive accuracy of evaluative techniques now in use, and provide a basis for determining the degree to which biological response is correlated with bioaccumulation of key contaminants in the species under study. The latter is an important aid in interpreting potential biological consequences of bioaccumulation. The program also meets EPA mission needs by providing an opportunity to document the application of a generic predictive hazard-assessment research strategy applicable to all wastes disposed in the aquatic environment. Therefore, the ERLN initiated exposure-assessment studies at the aquatic disposal site. The Corps-sponsored studies on environmental consequences of aquatic disposal will provide the effects assessment necessary to complement the EPA-sponsored exposure assessment, thereby allowing ERLN to develop and apply a hazard-assessment strategy. While not part of the Corps-funded FVP, the EPA exposure assessment studies will complement the Corps' work, and together the Corps and the EPA studies will satisfy the needs of both agencies.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Laboratory Evaluation of Adenylate Energy Charge as a Test for Stress in Mytilus edulis and Nephtys incisa Treated with Dredged Material"

5. In recognition of the potential national significance, the Office, Chief of Engineers, approved and funded the studies in January 1982. The work is managed through the Environmental Laboratory's Environmental Effects of Dredging Programs at WES. Studies of the effects of upland disposal and wetland creation are being conducted by WES and studies of aquatic disposal are being carried out by the ERLN, applying techniques worked out at the laboratory for evaluating sublethal effects of contaminants on aquatic organisms. These studies are funded by the Corps while salary, support facilities, etc., are provided by EPA. The EPA funding to support the exposure-assessment studies followed in 1983; the exposure-assessment studies are managed and conducted by ERLN.

6. The Corps and EPA are pleased at the opportunity to conduct cooperative research and believe that the value in practical implementation and improvement of environmental regulations of dredged material disposal will be considerable. The studies conducted under this program are scientific in nature and will be published in the scientific literature as appropriate and in a series of Corps technical reports. The EPA will publish findings of the exposure-assessment studies in the scientific literature and in EPA report series. The FVP will provide the scientific basis upon which regulatory recommendations will be made and upon which changes in regulatory implementation, and perhaps regulations themselves, will be based. However, the documents produced by the program do not in themselves constitute regulatory guidance from either agency. Regulatory guidance will be provided under separate authority after appropriate technical and administrative assessment of the overall findings of the entire program.

James Choromokos, Jr., Ph.D., P.E.
Director, Research and Development
U. S. Army Corps of Engineers

Bernard D. Goldstein, M.D.
Assistant Administrator for
Research and Development
U. S. Environmental Protection
Agency

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

| REPORT DOCUMENTATION PAGE | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|---|---|
| 1. REPORT NUMBER<br>Technical Report D-85-3 | 2. GOVT ACCESSION NO. A167374 3. RECIPIENT'S CATALOG NUMBER |
| 4. TITLE *(and Subtitle)*<br>LABORATORY EVALUATION OF ADENYLATE ENERGY CHARGE AS A TEST FOR STRESS IN *MYTILUS EDULIS* AND *NEPHTYS INCISA* TREATED WITH DREDGED MATERIAL | 5. TYPE OF REPORT & PERIOD COVERED<br>Final report<br>6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s)<br>Gerald E. Zaroogian, Carol Pesch, Paul Schauer, Diane Black | 8. CONTRACT OR GRANT NUMBER(s) |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS<br>US Environmental Protection Agency<br>Environmental Research Laboratory<br>Narragansett, Rhode Island 02882 | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS<br>Field Verification Program (Aquatic Disposal) |
| 11. CONTROLLING OFFICE NAME AND ADDRESS<br>DEPARTMENT OF THE ARMY, US Army Corps of Engineers, Washington, DC 20314-1000 and US Environmental Protection Agency, Washington, DC 20460 | 12. REPORT DATE<br>February 1985<br>13. NUMBER OF PAGES<br>56 |
| 14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*<br>US Army Engineer Waterways Experiment Station<br>Environmental Laboratory<br>PO Box 631, Vicksburg, Mississippi 39180-0631 | 15. SECURITY CLASS. *(of this report)*<br>Unclassified<br>15a. DECLASSIFICATION/DOWNGRADING SCHEDULE |

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

Available from National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161.

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Adenylate energy charge (WES)
Aquatic biology (LC)
Biological assay (LC)
Dredged material (WES)
Dredging (Biology) (LC)
Marine ecology--Research (LC)
Dredging--Environmental aspects--Evaluation (LC)

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Changes in adenine nucleotide metabolism were evaluated as indices of stress in the marine bivalve Mytilus edulis and the polychaete Nephtys incisa when treated with highly contaminated dredged material under laboratory conditions. Anesthetization of N. incisa is necessary to maximize the adenosine triphosphate (ATP) concentration and the adenylate energy charge (AEC) by eliminating all swimming activity and facilitating handling upon their removal from sediment. Extraction of adenine nucleotides from N. incisa

(Continued)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

20. ABSTRACT (Continued).

required the addition of ethylenediaminetetraacetic acid to perchloric acid to obtain consistently high recovery efficiencies of 96 ± 0.3 percent for ATP. Perchloric acid alone gave consistent recovery efficiencies of 92 ± 0.5 percent of ATP from adductor muscle tissue of M. edulis.

The AEC for M. edulis treated with 50 percent reference REF/50 percent Black Rock Harbor (BRH) dredged material was significantly different from all other treatments in test I (17 Nov 83). In test II (19 Mar 84) no significant differences occurred among treatments. At the end of treatment, M. edulis from the 50 percent REF/50 percent BRH had significantly lower AEC values than those AEC values obtained at the start of treatment. Reproducibility between tests was determined by comparison of the same treatment between tests for all treatments.

The AEC for M. edulis treated with 50 percent REF/50 percent BRH differed significantly between tests. No other significant differences occurred among the other treatments between tests with M. edulis. Although a significant difference occurred with the treatment 50 percent REF/50 percent BRH between tests, the data support the fact that the reproducibility of AEC for the same treatment between tests is excellent when variations in experimental conditions are considered.

The AEC for N. incisa treated with BRH/REF was significantly different from all other treatments within a test for test I (2 Sept 83) and test II (20 Sept 83). No other significant differences occurred among the other treatments in either test. Tests for reproducibility indicated that the AEC for N. incisa treated with REF/BRH differed significantly between tests. The biological significance of this difference is questionable. No other significant differences occurred among the other treatments.

Reproducibility within and between tests is exceptionally good for both M. edulis and N. incisa. Both M. edulis and N. incisa are excellent species with which AEC can be used to accurately assess their metabolic state and health condition when exposed to sublethal environmental perturbations.

This investigation is the first phase in developing field-verified bioassessment evaluations for the Corps of Engineers and the US Environmental Protection Agency regulatory program for dredged material disposal. This report is not intended for regulatory purposes; appropriate assessment methodologies that are field verified will be available at the conclusion of this program.

# PREFACE

This report describes work performed by the U.S. Environmental Protection Agency (EPA) Environmental Research Laboratory, Narragansett, R.I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program (FVP)). The FVP is sponsored by the Office, Chief of Engineers (OCE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES), under the purview of the Environmental Laboratory's (EL) Environmental Effects of Dredging Programs (EEDP). The OCE Technical Monitors for FVP were Drs. William L. Klesch and Robert J. Pierce. The objective of this program is to verify existing predictive techniques for evaluating the environmental consequence of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP study is being conducted by ERLN, with the wetland and upland portions conducted by WES.

The principal ERLN investigators for this aquatic study were Dr. Gerald Zaroogian, biochemist; Dr. Paul Schauer; Ms. Carol Pesch, research aquatic biologist; and Ms. Dianne Black, research aquatic biologist. Sample preparation and nucleotide analyses were conducted under the supervision of Dr. Zaroogian assisted by Ms. Mary Johnson. Laboratory exposures of N. incisa were performed under the supervision of Dr. Schauer and assisted by Dr. Gerald Pesch, Mr. John Sewall, and Mr. Michael Balboni. Ms. Dianne Black supervised the laboratory exposures of M. edulis with assistance from Ms. Melissa Hughes and

Mr. Greg Tracey. Data management and data analysis were conducted by Mr. Jeffery Rosen and Mr. James Heltsche.

The EPA Technical Director for the FVP was Dr. John H. Gentile; the Technical Coordinator was Mr. Walter Galloway; and the Project Manager was Mr. Allan Beck.

The study was conducted under the direct WES management of Drs. Thomas M. Dillon and Richard K. Peddicord, Contaminant Mobility and Regulatory Criteria Group (CMCG); Ecosystem Research and Simulation Division (ERSD); EL. Dr. C. Richard Lee was Chief, CMCG. Mr. Donald L. Robey was Chief, ERSD. Dr. John Harrison was Chief, EL. The FVP coordinator was Mr. Robert L. Lazor, and the EEDP Manager was Mr. Charles C. Calhoun, Jr.

Commander and Director of WES during preparation of the report was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

Zaroogian, G. E., et al. 1985. "Laboratory Evaluation of Adenylate Energy Charge as a Test for Stress in Mytilus edulis and Nephtys incisa Treated with Dredged Material," Technical Report D-85-3, prepared by US Environmental Protection Agency, Narragansett, R. I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

# EVALUATION OF ADENYLATE ENERGY CHARGE AS A TEST FOR STRESS IN MYTILUS EDULIS AND NEPHTYS INCISA TREATED WITH DREDGED MATERIAL

## PART I: INTRODUCTION

### Background

1. Historically, toxicity studies involving aquatic organisms have measured classical parameters such as growth, reproduction, and mortality. In most situations, these measures suffer from their lack of sensitivity. A biochemical marker or indicator of stress such as the adenylate energy charge (AEC) (Atkinson 1971) can be used to gain information on the physiological condition of an organism prior to the occurrence of irreversible changes. Since most biochemical systems react to specific stressors, a more generalized indicator such as AEC is advantageous in natural areas which may be influenced by the interaction of pollutants and environmental factors simultaneously.

2. Adenylate energy charge is an indication of the amount of energy available to an organism from the adenylate pool. It is calculated from measured concentrations of three adenine nucleotides, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP), which are integral to the energy metabolism of all organisms (Atkinson 1971). The AEC, defined as (ATP + 1/2 ADP)/ (ATP + ADP + AMP), has a maximum value of 1.0 when all adenylate is in the form of ATP and a minimum value of 0 when all adenylate is in the form of AMP (Atkinson and Walton 1967). The energy charge has been considered important in the control of key catabolic and anabolic pathways (Atkinson 1971).

Values of energy charge correlate with physiological condition: energy charges between 0.8 and 0.9 are typical of organisms which are actively growing and reproducing, usually under optimal environmental conditions (Atkinson 1971; Chapman et al. 1971; Ball and Atkinson 1975; Wiebe and Bancroft 1975; Chapman and Atkinson 1977; Karl and Holm-Hansen 1978; Rainer et al. 1979; Ivanovici 1980; Karl 1980; Giesy et al. 1981; Mendelssohn and McKee 1981; Romano and Daumas 1981; Skjoldal 1981; Dickson et al. 1982; Vetter and Hodson 1982; Zaroogian et al. 1982; Geisy et al. 1983; Hoya et al. 1983). Values in the range of 0.5 to 0.7 have been observed in organisms which are stressed (Ball and Atkinson 1975; Behm and Bryant 1975; Wiebe and Bancroft 1975; Wijsman 1976; Karl and Holm-Hansen 1978; Rainer et al. 1979; Christensen and Devol 1980; Ivanovici 1980; Karl 1980; Giesy et al. 1981; Mendelssohn and McKee 1981; Romano and Daumas 1981; Vetter and Hodson 1982; Zaroogian et al. 1982) and whose growth and reproduction rates are reduced (Chapman et al. 1971). Values below 0.5 have been associated with irreversible loss of viability under detrimental conditions (Ridge 1972; Montague and Dawes 1974; Ball and Atkinson 1975; Wijsman 1976; Karl and Holm-Hansen 1978; Skjoldal and Bakke 1978; Christensen and Devol 1980; Giesy et al. 1983; Vetter and Hodson 1982). If these relationships apply generally, a knowledge of the energy charge of key species with known responses to particular environmental conditions would provide a convenient measure to assess the extent to which these species are stressed.

## Objectives

3. The objective of this study was to evaluate the applicability of AEC as a measure of stress in M. edulis and N. incisa treated with highly contaminated dredged material under laboratory conditions and to determine the degree of variability and reproducibility inherent in the test. This objective is referred to as the Field Verification Program (FVP) and is the subject of this report.

## PART II: MATERIALS AND METHODS

### Overview

4. The types of tests conducted for AEC included both suspended particulate and solid phase exposures to Black Rock Harbor (BRH) sediments. Suspensions of either reference (REF) or BRH sediment were used in various combinations with a solid phase ranging from 100 percent REF to 100 percent BRH sediment where appropriate. Tests combining the solid and particulate phase were representative of the type of condition at the disposal site; however, the concentrations of suspended material used in the tests did not necessarily simulate actual field concentrations. Concentrations were chosen to produce a dose response in the endpoint measurements.

5. The tests described below generally follow methods prescribed in Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (ASTM 1980). Although the ASTM test methods were not specifically designed for sediment tests, they provide guidelines for experimental designs, water quality parameters, statistical analyses, and animal care, handling, and acclimation.

### Sediment Collection and Preservation

6. Reference sediment for these studies was collected from the FVP South reference site (40°7.95'N and 72°52.7'W), which is approximately 700 m south of the southern perimeter of the Central Long Island Sound (CLIS) disposal site (Figure 1). Reference sediment was collected with a Smith-McIntrye grab sampler (0.1 $m^2$) in August and December 1982 and May

1983 (collections I, II, and III, respectively). Sediment from each collection was returned to the laboratory, press sieved (wet) through a 2-mm mesh stainless steel screen, homogenized in a tub with a paddle, and stored in polypropylene (collection I) or glass (collections II and III) containers at 4°C until used in experiments. Each container of material was coded with collection number, date, and jar number.*

7. Black Rock Harbor sediment was collected from 25 locations within the highly industrialized Black Rock Harbor (Bridgeport, Conn.) study area with a 0.1-$m^2$ gravity box corer to a depth of 1.21 m (Figure 2). The sediment was homogenized, distributed to barrels, and stored at 4°C. The contents of each barrel were homogenized in a tub with a paddle, wet sieved through a 1-mm sieve, distributed to glass jars, and stored at 4°C until used in experiments. Samples of sediment were taken at various points in the collection, mixing, and distribution procedure for moisture content and chemical analysis.

---

* See Lake et al. (1984) for complete details.

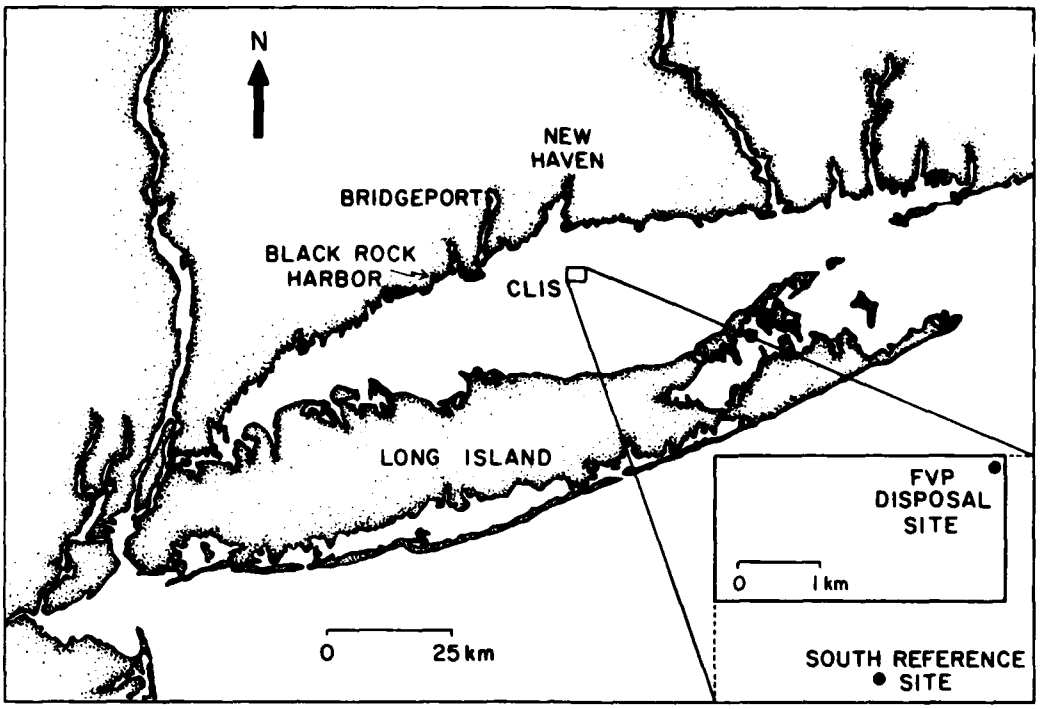



Figure 1. Central Long Island Sound disposal site and South reference site

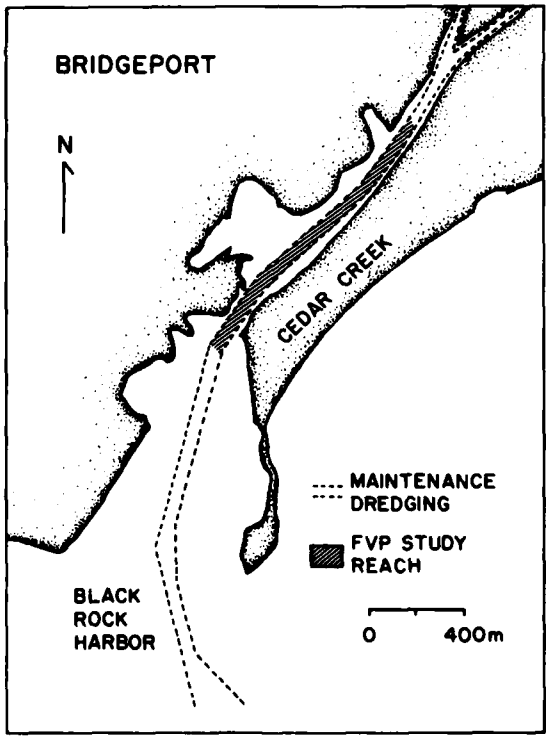



Figure 2. Black Rock Harbor, Connecticut, source of dredged material

## Mytilus edulis

### Collection and Holding

8. Mytilus edulis were collected with a scallop dredge from an uncontaminated site near Dutch Island in the West Passage of Narragansett Bay, R.I. (71°24.0'W and 41°29.4'N) from depths ranging between 5 m and 10 m. Collection information for each test is listed below:

| Test | Collection Date | Seawater Temperature °C | Salinity(ppt) | Testing Initiated |
|---|---|---|---|---|
| I | 10 Nov 83 | 13 | 31 | 17 Nov 83 |
| II | 9 Mar 84 | 5 | 29 | 19 Mar 84 |

The mussels were sorted to obtain a size range of 50 to 55 mm shell length and held in a laboratory flow-through system with unfiltered seawater at ambient temperature. Acclimation, if necessary, was conducted in running unfiltered seawater at a rate of 1°C per day to 15°C as this was the temperature selected for testing.

### Exposure Methods

9. Since 50 mg/ℓ of suspended REF sediment did not adversely affect M. edulis during 28 days exposure, this concentration of particulate was selected as the no-observable-effect-concentration. Therefore, 50 mg/ℓ was used as the suspended solids concentration in all tests. Two experiments were designed to examine the effects of BRH dredged material on M. edulis (Lake et al. 1984). The experimental design included three concentrations of particulate exposure in the following ratios: 100 percent REF/0 percent BRH, 50 percent REF/50 percent BRH, and 0 percent REF/100 percent BRH. The measured

concentrations are given in Table 1. The exposure conditions were chosen on the basis of previous experiments and were expected to be sublethal for the 28-day exposure period. Survival of M. edulis was 100 percent in all treatments except for the 100 percent BRH and the 50 percent REF/50 percent BRH treatments in test I (Table 1).

10. Figure 3 illustrates the M. edulis exposure system and Figure 4 illustrates the sediment dosing system. In the conduct of tests with M. edulis, the REF and BRH mixing and distribution chambers (Figure 3) were maintained at 50 mg/ℓ and treatment combinations were obtained by proportionally siphoning suspended sediment from the appropriate distribution chambers to produce a combined flow of 300 mℓ/min in each exposure chamber. On day 0 and 28, M. edulis were sampled for AEC. Test I was terminated on day 26 instead of day 28 since the reduction in feeding, especially in the 100 percent BRH treatment, which was observed to be quite significant on day 26, could have indicated a rapidly deteriorating health condition, which in turn could have resulted in death prior to 28 days. Therefore, it was thought prudent to terminate at 26 days.

11. Spectrophotometric measurements of the amount of suspended particulates entering the exposure chambers were made daily using the relationship between absorbance and dry weight of suspended particulates. The latter was determined by collecting triplicate samples of suspended sediment directly from the diluter or by preparing serial dilutions from the highest concentration. The dry weight of these samples was measured using the methods reported in Lake et al. (1984). Linear

Table 1

Measured Concentrations (Dry Weight) of Suspended Particles for Tests with M. edulis

| Treatment | Concentration (mg/ℓ) Added to Chamber $\bar{x} \pm SD$ | Concentration (mg/ℓ) In Chamber $\bar{x} \pm SD$ | Concentration Range In Chamber (mg/ℓ) | Death %* |
|---|---|---|---|---|
| | | Test I 17 Nov 83 | | |
| 100% BRH | 62.8 ± 9.9 | 30.2 ± 17.5 | 73.4 - 5.0 | 0 |
| 100% REF | 56.2 ± 8.2 | 11.6 ± 5.1 | 24.3 - 2.0 | 3 |
| 50%REF/50%BRH | 59.4 ± 5.5 | 24.5 ± 15.4 | 63.3 - 9.4 | 13 |
| | | Test II 19 Mar 84 | | |
| 100% BRH | 56.2 ± 8.6 | 29.1 ± 11.4 | 48.6 - 5.0 | 0 |
| 100% REF | 49.4 ± 6.1 | 14.1 ± 6.4 | 28.9 - 6.4 | 0 |
| 50%REF/50%BRH | 52.9 ± 5.7 | 23.5 ± 10.1 | 45.4 - 6.4 | 0 |

*Based on a total of 40 mussels per treatment.

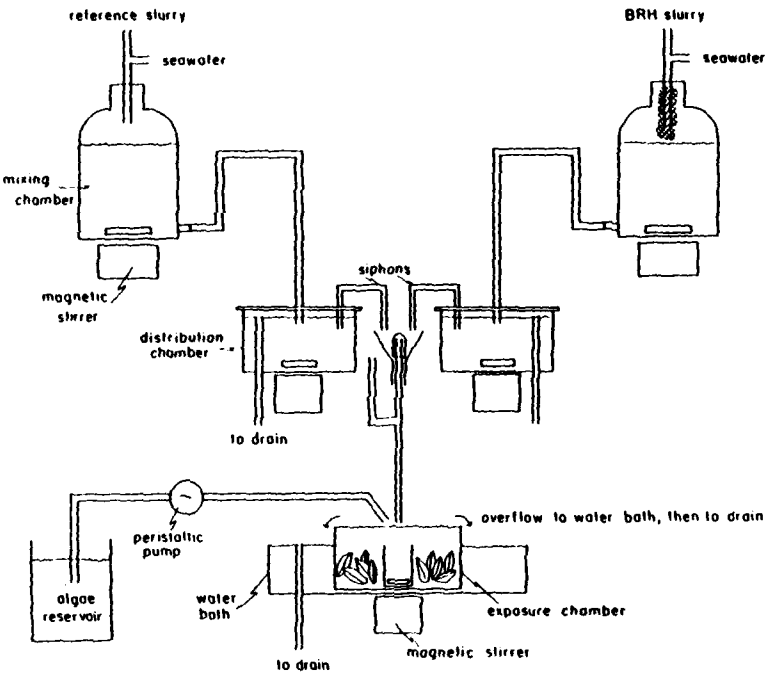



Figure 3. Schematic of the dosing system used to treat M. edulis with various concentrations of BRH and REF sediment suspensions

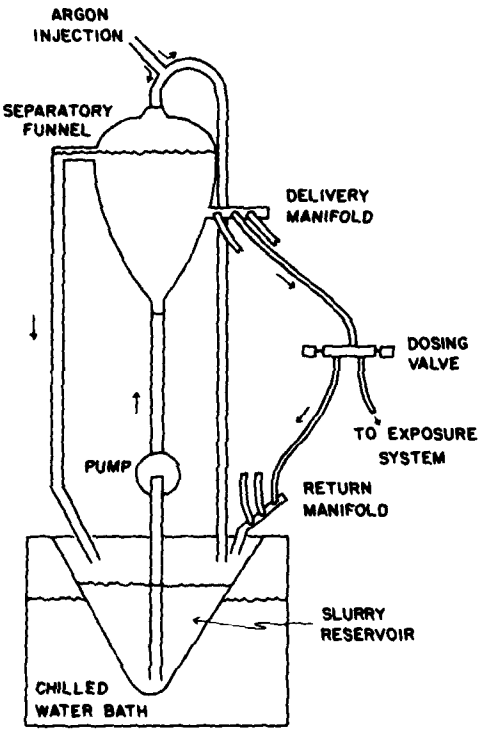



Figure 4. Sediment dosing system with chilled water bath and argon gas supply

regression analysis of the data established the relationship between absorbance at 660 nm and dry weight. Analysis of variance and multiple comparison tests were performed on the suspended particulate data collected daily during the experiment.

12. Forty M. edulis were continuously fed laboratory-cultured Isochrysis galbana at a rate of 94 mg (dry weight) per mussel per day. Conditions and techniques of algal culture were modified after Guillard (1975). Guillard's "f/2" nutrient media was used, except that all trace metals but iron were eliminated and the concentration of the vitamins thiamin and B12 were doubled.

Adenylate Extraction

13. The adductor muscle was rapidly dissected out, blotted dry, placed on a labelled polythene strip (Gladwrap®), and freeze clamped with aluminum blocks cooled to -196°C with liquid nitrogen (Ivanovici 1980; Bergmeyer 1965). The time between sampling and dissection never exceeded 10 minutes. Tissue samples were removed and freeze clamped in less than 30 sec and the labelled samples were stored in liquid nitrogen until homogenization.

14. Adenine nucleotides were extracted from tissues with a method similar to that of Ivanovici (1980) (Figure 5). The freeze-clamped tissue was quickly transferred from its wrapping to a tared stainless steel homogenizing tube previously cooled in liquid nitrogen and placed in a polyurethane insulator and weighed. Tissue samples (approx. 0.2 g) were ground to a fine powder at -196°C. Perchloric acid (PCA) (1 ml, 6% v/v) was added to the ground tissue and allowed

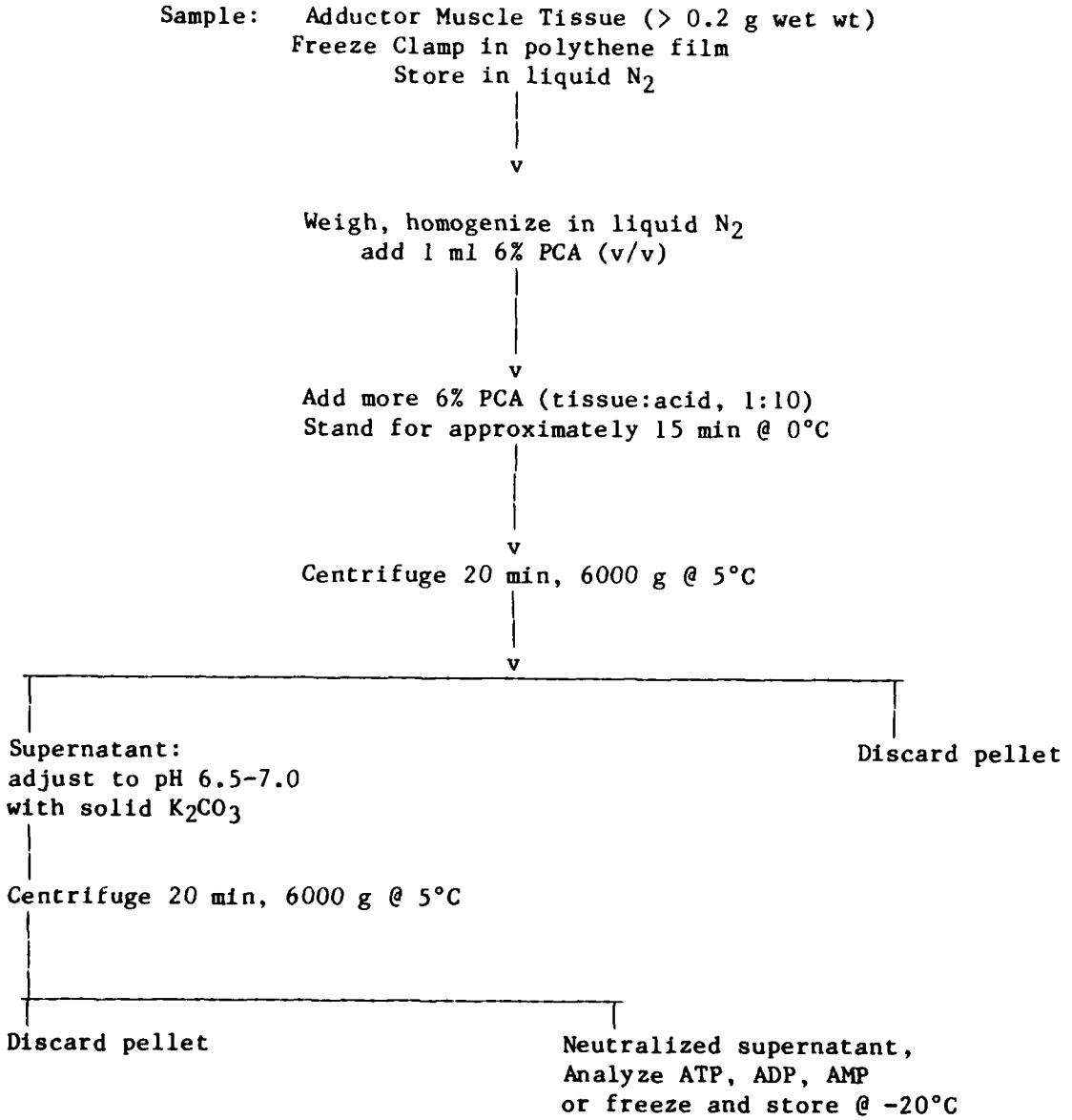



Figure 5. Summary of the procedure for the extraction of adenine nucleotides from the adductor muscle of M. edulis

to freeze, ground to a powder, and mixed with the tissue sample. This mixture was kept on ice and allowed to thaw, after which additional ice-cold PCA was added (the final ratio of tissue to PCA was 1:10, w/v) and then centrifuged at 5°C and 6000 g for 20 min after thorough mixing. The supernatant was decanted into a (polyethylene) centrifuge tube containing 5 µl of Universal indicator and adjusted to pH 6.5-7.0 with solid $K_2CO_3$. These tubes were left on ice for approximately 15 min to allow $CO_2$ evolution and then centrifuged as above. The supernatant was decanted from the $KClO_4$ precipitate into clean (polyethylene) centrifuge tubes and assayed or stored at -20°C. Generally 20 samples were prepared each day. Recovery efficiency of the extraction was determined by spiking tissue samples with ATP, ADP, and AMP and recovery was calculated by the following equation:

$$\% \text{ recovery} = \frac{[\text{Sample + Standard}] - [\text{Sample}]}{[\text{Standard}]} \times 100\% \qquad (1)$$

where

Sample + Standard = concentration of adenylates in sample spiked with adenylates

Sample = concentration of adenylates in sample

Standard = concentration of adenylate standard

15. Extraction efficiences of adenine nucleotides from adductor muscle tissue of M. edulis by PCA were consistently greater than 92 ± 0.5 percent. It is important to note that the extraction efficiencies

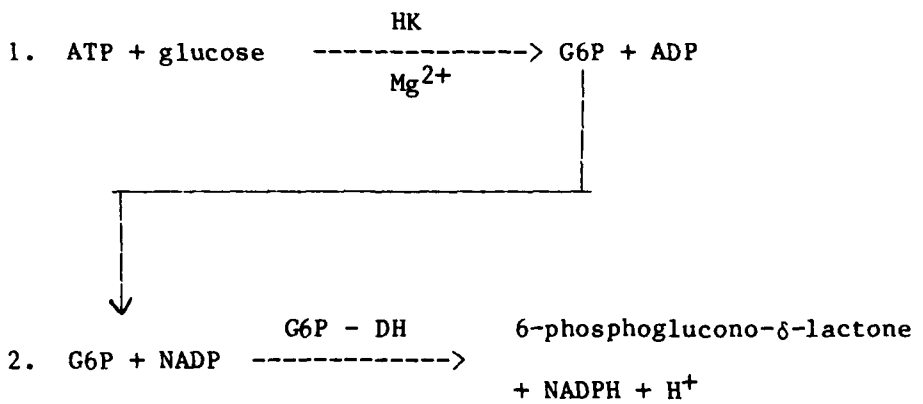
refer to the extraction procedure and do not necessarily reflect intracellular extraction efficiencies.

Adenylate Assay

16. Upon thawing, frozen samples were centrifuged as above to remove any $KClO_4$ as precipitate before assaying for adenylates.

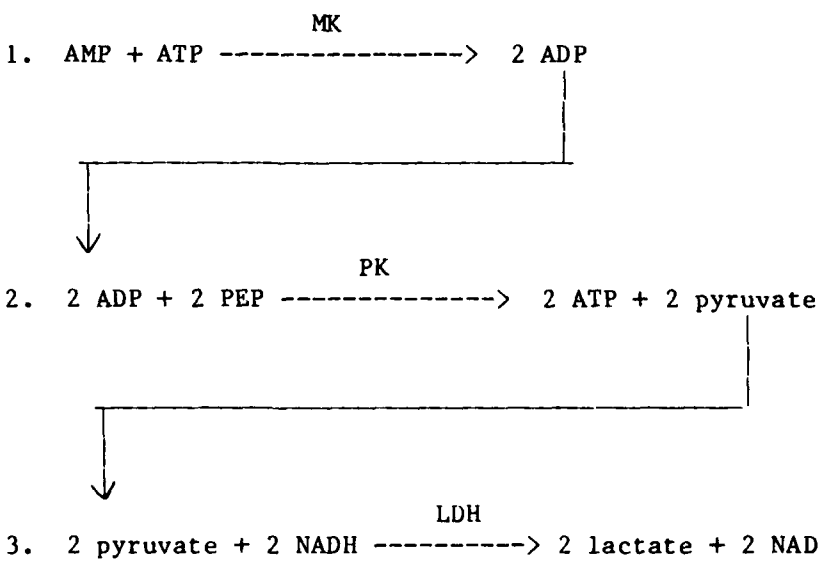
17. The concentrations of ATP, ADP, and AMP were determined spectrophotometrically (340 nm) with hexokinase (Lamprecht and Trautschold 1974), pyruvate kinase, and myokinase (Adam 1963), respectively (Figure 6). All enzymes, chemicals, and reagents (analytical grade) were obtained from Boehringer Mannheim, Indianapolis, Indiana.

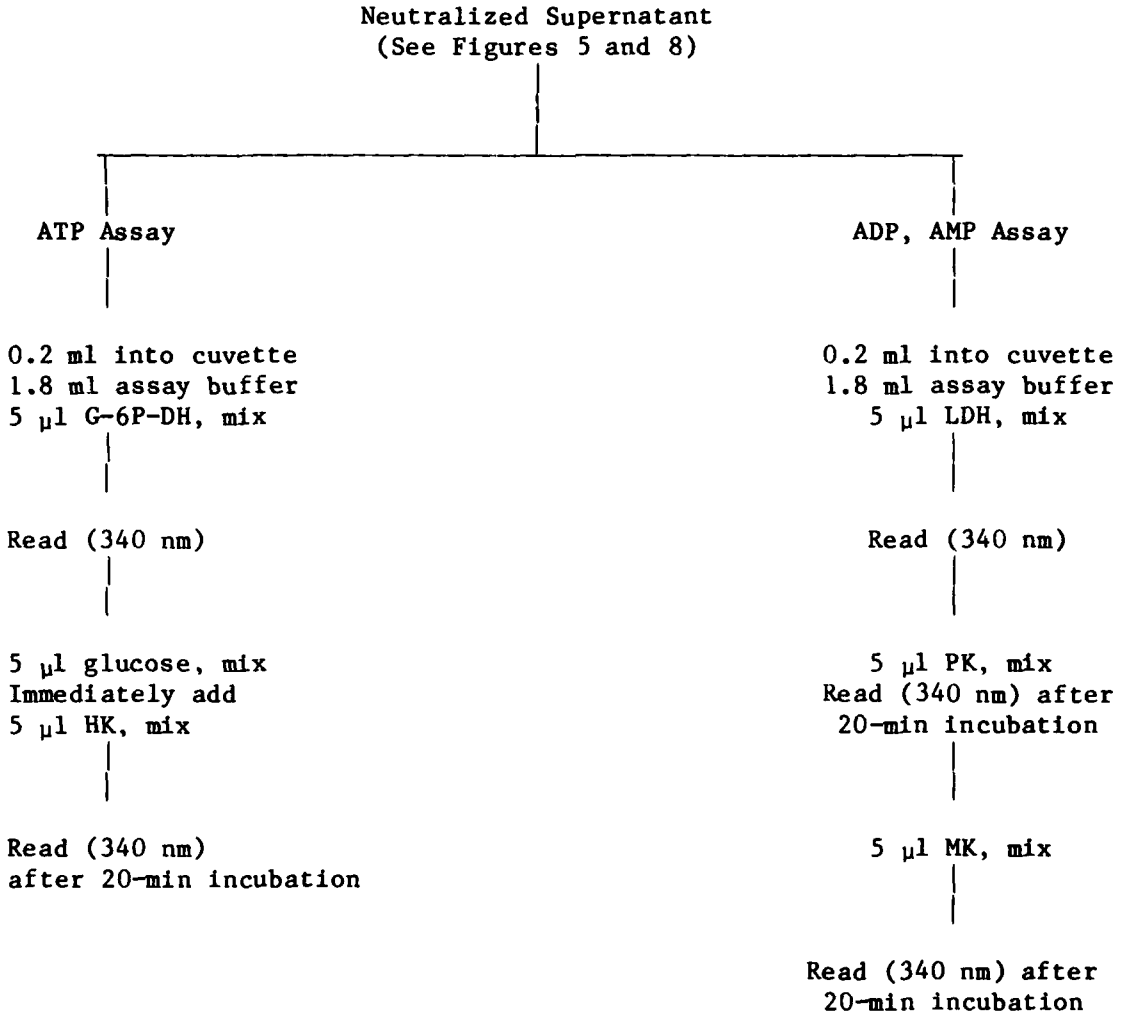
18. The principle of the ATP assay is as follows: glucose is phosphorylated by ATP to glucose-6-phosphate (G6P) with hexokinase (HK) (reaction 1). Glucose-6-phosphate then reacts with nicotinamide-adenine dinucleotide phosphate (NADP) to form 6-phosphoglucono-δ-lactone and reduced nicotinamide-adenine dinucleotide phosphate (NADPH). This reaction is catalyzed by glucose-6-phosphate dehydrogenese (G6P-DH) (reaction 2).



Thus for every micro-mole of ATP, one micro-mole of NADPH is formed and causes an increase in absorbancy at 340 nm.

19. The principle of the ADP and AMP assays is as follows: pyruvate kinase (PK) catalyzes the phosphorylation of one micro-mole of ADP by phosphoenolpyruvate (PEP) to form one micro-mole of ATP and pyruvate (reaction 2). Pyruvate in turn is converted to lactate by lactate dehydrogenase (LDH). Thus, one micro-mole of ADP results in the formation of one micro-mole of nicotinamide-adenine dinucleotide (NAD) (reaction 3). The decrease in absorbancy at 340 nm caused by the formation of NAD from NADH is, therefore, proportional to the amount of ADP present in the sample. After this absorbance change has been measured in a sample, myokinase (MK) is added. This enzyme catalyzes the formation of 2 micro-moles of ADP from one micro-mole each of AMP and ATP (reaction 1). In turn, 2 micro-moles of NAD are formed (reactions 2 and 3).






G-6P-DH - glucose-6-phosphate dehyrogenase

HK - hexokinase

LDH - lactate dehydrogenase

PK - pyruvate kinase

MK - myokinase

Figure 6. Summary of the procedures for analysis of ATP, ADP, and AMP in adductor muscle tissue of M. edulis and whole N. incisa

20. To determine if any inhibitory effects of neutralized tissue extracts on the nucleotide assay system occurred, known amounts of ATP, ADP, and AMP were added to neutralized extracts as internal standards and assayed to check for inhibitory or enhancement effects by the extract. The following equations were used to calculate correction factors (Cf):

$$X\% = \frac{[\text{Sample + Internal Standard}] - [\text{Sample}]}{[\text{Internal Standard}]} \quad (2)$$

$$Cf_{\text{ATP, ADP or AMP}} = \frac{100\%}{100\% + X\%} \quad (3)$$

21. A correction factor was not required for ATP since extracts of M. edulis adductor muscle had a negligible effect on absorbance. However, these same extracts increased absorbance which caused over readings for ADP (112 percent) and AMP (111 percent). Thus, a correction factor was required for ADP (0.89) and AMP (0.90) to calculate accurately their concentration.

## Nephtys incisa

### Collection, Culture, and Holding

22. Nephtys incisa is a marine polychaete worm which is indigenous to the disposal area in Central Long Island Sound (CLIS). Worms were collected with a Smith-McIntyre grab sampler (0.1 $m^2$) from the South reference site (Figure 1) at various times in 1983 prior to the test

periods and held in the laboratory for a short acclimation period.* Tests were conducted with worms 3 to 4 cm in length.

Exposure Methods

23. Two 10-day suspended particulate tests (same treatments, performed at different times) with separate collections of N. incisa were performed.

24. The suspended sediment experimental system consisted of three modules: the controlled dosing system, the dilution and distribution system, and the test chambers (Figures 4 and 7). Two identical dosing systems, one for REF and one for BRH, provided a constantly recirculating source of concentrated sediment slurry (in seawater) passing by a three-way valve, that led to the dilution and distribution system. Argon gas was added to the reservoir of the dosing system to minimize oxidation of the slurry (Figure 4). The three-way valve was controlled by a microprocessor programmed to deliver a pulse of slurry at periodic intervals. In the dilution and distribution system, the concentrated slurry was mixed with seawater to the proper concentration of suspended solids and distributed to the individual test chambers. Actual concentration of suspended particulates in the test chambers was determined (by dry weights) periodically.*

25. The test chambers were glass crystallizing dishes (150 by 75 mm), which contained 400 ml of sediment (2.5 to 3.5 cm deep). Each dish contained a smaller glass crystallizing dish (60 by 35 mm) in the

---

* See Lake et al. (1984) for complete details.

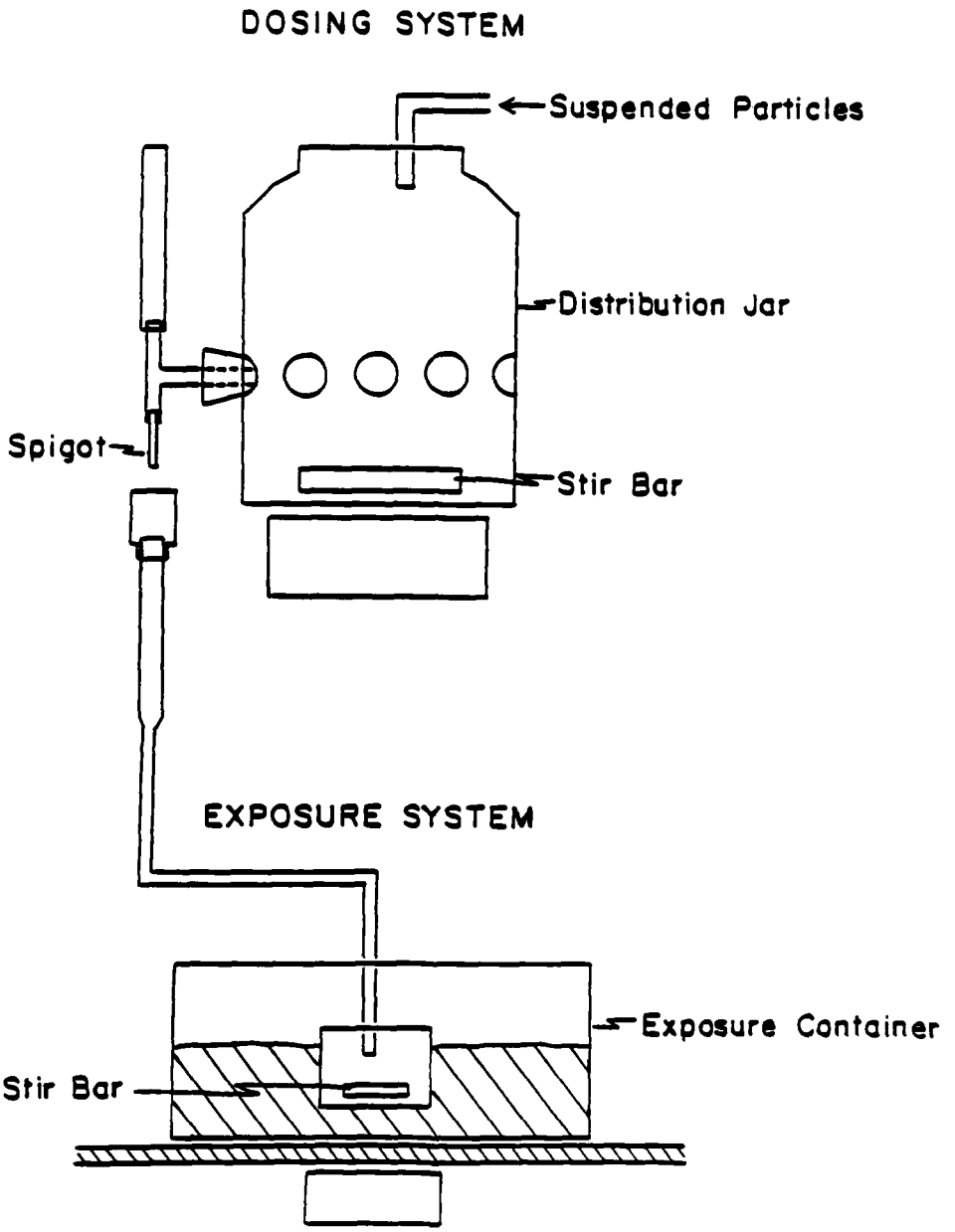



Figure 7. Suspended sediment dilution system, distribution chamber, and exposure chamber used for acute toxicity tests with N. incisa

center of the larger dish. A Teflon®-coated stir bar was placed in the small dish, which received the inflow water, to keep the particulate material in suspension. The inflow water flowed out of the central dish over the sediment surface, and overflowed the edge of the large crystallizing dish.

26. Exposure conditions for the solid phase portion of the suspended particulate tests were 100 percent REF or 100 percent BRH sediment. These two solid phase exposure conditions in combination with the two suspended sediment exposures, REF or BRH at a nominal concentration of 200 mg/l (dry weight), gave a total of four treatments. The measured concentrations are given in Table 2. The exposure conditions for these experiments were chosen on the basis of previous experiments and were expected to be sublethal for the 10-day exposure period. Survival of N. incisa was 100 percent in all treatments except the BRH/REF treatment in the first test (Table 2).

27. The worms were fed prawn flakes (ADT-Prime, Aquatic Diet Technology, Brooklyn, N.Y.) in a suspension of seawater, which was pumped by peristaltic pump into the distribution chamber of the dosing system. The amount fed was 127 mg (dry weight) per test chamber per day. This amount of food was determined optimum in prior feeding studies with N. incisa.*

28. During the tests, all dishes were examined daily for the appearance of any worms on the surface of the sediment, but none were seen.

---

* Personal communication, Paul Schauer, March 1983, U.S. Environmental Protection Agency.

Table 2

Measured Concentrations (Dry Weight) of Suspended Particles for Tests with N. incisa

| Treatment suspended/solid | n* | Concentration (mg/ℓ) $\bar{x} \pm SD$ | % Dead** |
|---|---|---|---|
| | | Test I 02 Sept 83 | |
| REF/REF | 10 | 211 ± 87 | 0 |
| BRH/REF | 10 | 171 ± 53 | 3 |
| REF/BRH | 10 | 211 ± 87 | 0 |
| BRH/BRH | 10 | 171 ± 53 | 0 |
| | | Test II 20 Sept 83 | |
| REF/REF | 10 | 199 ± 73 | 0 |
| BRH/REF | 9 | 226 ± 47 | 0 |
| REF/BRH | 11 | 199 ± 73 | 0 |
| BRH/BRH | 12 | 226 ± 47 | 0 |

* Number of worms analyzed for AEC from a total of 30 worms; the remainder were used for other purposes.

** Based on a total of 30 worms per treatment.

On the last day of the test, observations were made on the burrows visible through the sides of the dishes, and the depth of the suspended material deposited on top of the solid phase was measured. Worms missing were presumed dead. These results are reported elsewhere.

29. All tests were conducted with sand-filtered Narragansett Bay seawater at 20°C and approximately 30 ppt salinity. Flow rates were about 35 ml/min. The photoperiod was a 14:10 hr light-dark cycle. Nephtys incisa were acclimated in REF sediment for a minimum of five days at 20°C.

Adenylate Extraction

30. The worms from each treatment replicate were collected on a fine mesh sieve (0.9 mm mesh) and immediately anesthetized by immersion of sieve and worms into a 7 percent solution of $MgCl_2$ in seawater for 2-1/2 min (Dean and Mazurkiewicz 1975). The worms were washed by immersion of the sieve in clean seawater and the worms were removed from the sieve and placed into a Carolina dish (75 mm diam.) containing approximately 50 ml clean seawater. One or two anesthetized worms (>0.1 g wet wt.) were placed on a millipore filter pad (25 mm, 1.2 μ) and as much seawater as possible was removed by vacuum. The anesthetized worms were gently removed from the filter pad onto a labelled polythene strip and freeze clamped (Figure 8). The extraction procedure was identical to that used for nucleotide extraction from M. edulis except that the homogenized tissues were doubly extracted with 6 percent PCA containing 0.33 percent ethylenediaminetetraacetic acid (w/v) (EDTA) and the tissue extracts were assayed within 2 hr of extraction.

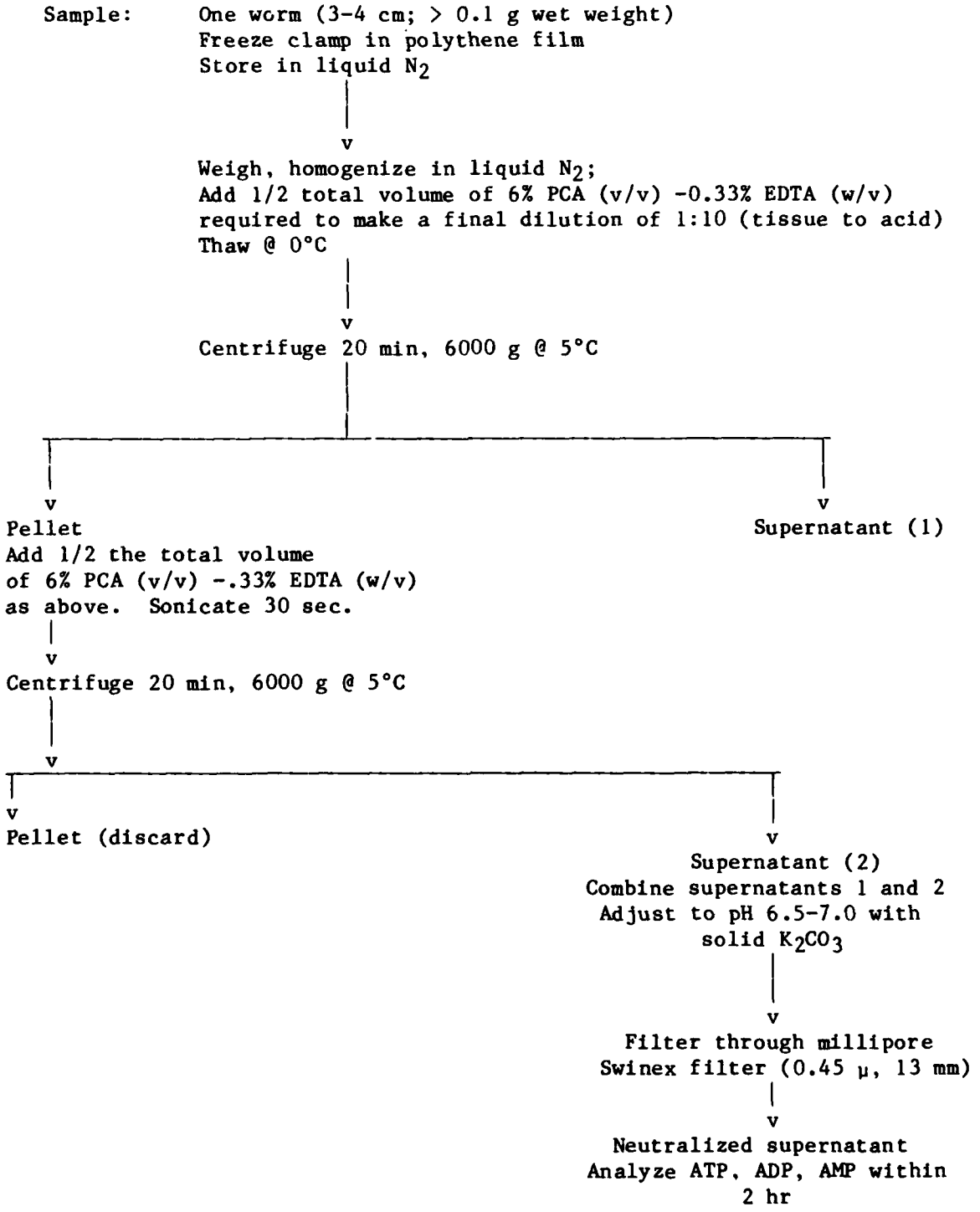



Figure 8. Summary of the procedure for the extraction of adenine nucleotides from the marine polychaete N. incisa

The double extraction of whole N. incisa with PCA-EDTA gave extraction efficiencies consistently greater or equal to 96 percent ± 0.3 percent.

Adenylate assay

31. The assay procedure for N. incisa extracts was identical to that for M. edulis (Figure 6).

32. Inhibitory effects by the extracts of N. incisa on ATP analysis were negligible thus requiring no correction factor. However, these same extracts increased absorbance which caused overreadings for ADP (111 percent) and AMP (109 percent). Thus a correction factor (Cf) was required for ADP (0.90) and AMP (0.91) to calculate accurately their concentration.

## Statistical Analyses

33. Means and standard error were calculated for the concentrations of the individual adenine nucleotides and the AEC. Sample size determinations were based upon a predetermined type I error of 0.05 and a type II error of 0.20 with fixed differences of 0.1 or 0.05 unit for AEC. Non-pooled data were analyzed with analysis of variance (ANOVA) methods to detect differences and determine the reproducibility of AEC among treatments within a test. If significant differences in AEC were indicated by the ANOVA, then Tukey's (HSD) test for pairwise comparison of means between treatments within a test was used. The reproducibility between tests was determined with ANOVA by comparing the same treatment between tests for all treatments. This was followed by Tukey's test if the ANOVA indicated significant differences.

## PART III: RESULTS

### Mytilus edulis

### Extraction

34. Extraction procedures used in this and a previous study (Zaroogian et al. 1982) for M. edulis adductor muscle tissue provided high recovery efficiencies of 92 ± 0.5 percent. Low variability among AEC values within treatments is evident in the small sample size required to detect a 0.05 change in AEC values (Table 3).

Table 3

Sample Size Determinations for the Detection of Fixed Magnitudes of Differences in AEC for M. edulis after being Treated with BRH Sediment under Laboratory Conditions for 26 Days (Test I) and 28 days (Test II)

| Variable | Magnitude of difference | Variance | N* |
|---|---|---|---|
| | Test I 17 Nov 83 | | |
| AEC | 0.1 | 0.00176 | 3 |
| AEC | 0.05 | 0.00176 | 9 |
| | Test II 19 Mar 84 | | |
| AEC | 0.1 | 0.00159 | 2 |
| AEC | 0.05 | 0.00159 | 8 |

* N - Calculated sample size needed to detect a 0.1 or 0.05 unit change in AEC. The power of the test = 0.80; $\alpha = 0.05$ (Snedecor and Cochran 1980).

Adenylate Energy Charge

35. Sample size determinations which were calculated with estimates of variation indicated that sample sizes of 9 would allow detection of a significant difference in AEC of 0.05. Whereas, sample sizes of 3 are required to detect a 0.1 difference in AEC (Table 3). Also apparent in Table 3 is the excellent reproducibility in sample size requirements between experiments. This is due to the similarity in variance in the two tests (Table 3). Analyses of variance coupled with Tukey's test for grouping of means indicated that the AEC for the treatment 50 percent REF/50 percent BRH is significantly different from all other treatments in test I (17 Nov 83) (Table 4). In test II (19 Mar 84) these same statistical analyses indicated that no significant differences occurred among treatments (Table 4). At the end of the treatment period, M. edulis from all the treatments had lower AEC values than the AEC values obtained at the start of treatments in each test; however, the difference was statistically significant only for the 50 percent REF/50 percent BRH treatment in both tests (Table 4). In order to determine the reproducibility between tests, analyses of variance coupled with Tukey's test were performed by comparison of the same treatment between tests for all three treatments. It is evident in Table 4 that the AEC for the 50 percent REF/50 percent BRH treatment differed significantly between tests. No other significant difference occurred among the other treatments between tests (Table 4). Although a significant difference occurred with the 50 percent REF/50 percent BRH treatment between tests, the data support the fact that the reproducibility of AEC for the same treatment

between tests is excellent when variations in experimental conditions are considered (Tables 1 and 4).

Table 4

The Response of Adenine Nucleotides in Adductor Muscle Tissue of M. edulis after Treatments with BRH Sediment for 26 days (Test I) and 28 Days (Test II) under Laboratory Conditions

| | | µmol/g Wet Weight Tissue | | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | n | ATP | ADP | AMP | AEC | | |
| | | Test I 17 Nov 83 | | | | | |
| Time 0* | 9 | 3.63(0.16)** | 0.93(0.05)** | 0.14(0.01)** | 0.87(0.01)** | A† | |
| 100% REF | 10 | 2.86(0.09) | 1.07(0.08) | 0.16(0.03) | 0.83(0.01) | A | |
| 100% BRH | 10 | 2.70(0.13) | 0.97(0.06) | 0.16(0.04) | 0.83(0.01) | A | |
| 50%REF/50%BRH | 10 | 2.38(0.11) | 1.22(0.04) | 0.36(0.04) | 0.75(0.01) | B | a†† |
| | | Test II 19 Mar 84 | | | | | |
| Time 0* | 10 | 3.35(0.19)** | 0.93(0.05)** | 0.08(0.01)** | 0.87(0.01)** | A† | |
| 100% REF | 10 | 3.53(0.15) | 1.21(0.07) | 0.19(0.03) | 0.83(0.01) | AB | |
| 100% BRH | 10 | 3.36(0.19) | 1.23(0.08) | 0.17(0.03) | 0.83(0.01) | AB | |
| 50%REF/50%BRH | 10 | 3.30(0.21) | 1.35(0.03) | 0.25(0.04) | 0.80(0.01) | B | b†† |

* Start of treatment.
** Mean value of each sample with standard error of mean in parentheses.
† Means with different letters differ significantly within a test at $\alpha = 0.05$.
†† Means of similar treatments with different letters differ significantly between tests at $\alpha = 0.05$ and means with no letter do not differ significantly.

## Nephtys incisa

### Anesthetization

36. Since preliminary AEC values were consistently low in N. incisa extracts, possible ways were sought to increase them (Tables 5 and 6). Initially, during the collection procedures, the worms were extremely active which made handling them difficult once the sediment was removed. It was thought that this activity was depleting the energy reserves and causing low AEC values. In order to eliminate this activity and facilitate handling, N. incisa were anesthetized. Anesthetization, which was accomplished by immersion of worms in a 7 percent magnesium chloride solution for 2 1/2 min, had a significant ($\alpha = 0.05$) effect on AEC (Table 5). These conditions of anesthetization allowed handling of worms without movement until they were freeze clamped. Worms were collected at the South reference site in Long Island Sound on 14 November 1982 and anesthetized and freeze clamped on board the vessel at the time of collection. In addition, anesthetization was not lethal to the worms since recovery occurred within 20 min after immersion of the worms in clean seawater.

Table 5

Adenylate Energy Charge and Adenine Nucleotide Concentrations in N. incisa with and without Anesthetization with Magnesium Chloride (n = 8)

| | μmol/g Wet Weight of Tissue | | | |
|---|---|---|---|---|
| Treatment | AEC | ATP | ADP | AMP |
| With Magnesium chloride | 0.64(0.01)A* | 1.02(0.13) | 0.65(0.01) | 0.06(0.03) |
| Without Magnesium chloride | 0.50(0.01)B | 0.33(0.13) | 1.36(0.03) | 0.26(0.01) |

* Means with different letters are significantly different at $\alpha$ =0.05.

37. The data in Table 5 would also indicate that worms not treated with magnesium chloride were under stress since a charge of 0.50 was obtained along with a decrease in the concentration of ATP with corresponding concentration increases of ADP and AMP. The AEC was higher in anesthetized worms since all swimming activity was eliminated and energy was conserved. Although the AEC values increased with anesthetization, they were not sufficiently high to indicate that the worms were not under stressful conditions (Table 5). This suggested that the extraction of nucleotides from N. incisa was unsatisfactory or a PCA-resistant ATP hydrolyzing enzyme (ATPase) was present in N. incisa.

Extraction

38. Neutralized tissue homogenates were analyzed for adenine nucleotides after storage at -20°C. A loss of ATP was observed after 12 hr storage. This indicated that a PCA-resistant ATPase was present in

N. incisa. Thus, a series of experiments were initiated to test trichloroacetic acid (TCA) at different concentrations as an extractant for adenine nucleotides from N. incisa.

39. Six freeze-clamped wafers, each containing 25 individual N. incisa, were broken into many small pieces while frozen. The pieces of each wafer were divided into two or three aliquots to give a tissue mass of approximately 0.2 g for each aliquot. Each aliquot of the same wafer was treated as a replicate for a particular treatment. Thus, there were six replicates with approximately the same worm mass for each treatment. This enabled a comparison with the same worm mass between treatments and eliminated a major source of variability inherent when using individuals. The data in Table 6, series 1, indicate that 6 percent PCA (v/v) is a better extractant of adenine nucleotides from N. incisa than 7 percent TCA (v/v), which is reflected in the higher AEC value. Next, the concentration of TCA was increased to 10 and 20 percent (v/v) and no difference was obtained between 6 percent PCA (v/v) and 10 or 20 percent TCA (v/v) for extraction of adenine nucleotides from N. incisa (Table 6, series 2). Extraction of adenine nucleotides from N. incisa tissue homogenates twice with 6 percent PCA (v/v) containing 0.33 percent EDTA (w/v) gave much better results than extractions twice with 6 percent PCA (v/v) (Table 6, series 3).

Table 6

Comparison of Perchloric and Trichloroacetic Acids for Extraction of Adenine Nucleotides from N. incisa for AEC Determinations

| Test Series | Treatment | Sample Size N | Adenine Nucleotides µmol/g Wet Weight Tissue ATP | ADP | AMP | AEC |
|---|---|---|---|---|---|---|
| 1 | PCA 6% v/v | 6 | 0.64(0.18)* | 0.36(0.05)* | 0.08(0.009)* | 0.73(0.01)* |
| | TCA 7% v/v | 6 | 0.10(0.03) | 0.19(0.03) | 0.03(0.005) | 0.49(0.01) |
| 2 | PCA 6% v/v | 6 | 0.38(0.12)* | 0.32(0.03)* | 0.06(0.005)* | 0.66(0.01)* |
| | TCA 10% v/v | 6 | 0.41(0.11) | 0.44(0.06) | 0.12(0.007) | 0.64(0.01) |
| | TCA 20% v/v | 6 | 0.51(0.08) | 0.49(0.06) | 0.13(0.02) | 0.59(0.01) |
| 3 | PCA 6% v/v | 6 | 0.19(0.04)* | 0.80(0.01)* | 0.14(0.04)* | 0.53(0.01)* |
| | (PCA 6% v/v) + (EDTA 0.33% w/v) | 6 | 2.05(0.48) | 0.79(0.07) | 0.14(0.005) | 0.81(0.01) |

* Mean value of each sample with standard error of the mean in parentheses.

40. Analysis of adenine nucleotides within 2 hr after extraction was another precaution taken for the preservation of adenine nucleotides during their extraction from N. incisa tissue. This precaution appeared to be necessary with N. incisa but not with M. edulis since neutralized PCA extracts could be stored for 4 weeks at -20°C with no apparent loss in adenine nucleotide concentration.

Adenylate Energy Charge

41. Since extraction of N. incisa with PCA-EDTA gave consistently high recovery efficiencies of 96 ± 0.3 percent, PCA-EDTA was selected as the method of choice for extraction of adenine nucleotides from N. incisa. In addition, low variability among AEC values within treatments is also apparent with the small sample size required to detect a 0.05 difference in AEC values (Table 7).

Table 7

Sample Size Determination for the Detection of Fixed Magnitudes of Differences in AEC for N. incisa After Being Treated for 10 Days with BRH Sediment Under Laboratory Conditions

| Variable | Magnitude of Difference | Variance | N* |
|---|---|---|---|
| | | Test I 02 Sept 83 | |
| AEC | 0.1 | 0.00091 | 2 |
| AEC | 0.05 | 0.00091 | 7 |
| | | Test II 20 Sept 83 | |
| AEC | 0.1 | 0.00106 | 2 |
| AEC | 0.05 | 0.00106 | 6 |

*N - Calculated sample size needed to detect a 0.1 or 0.05 unit change in AEC. The power of the test is 0.80; $\alpha$ = 0.05 (Snedecor and Cochran 1980).

42. As indicated in Table 7, a sample size of 7 would allow detection of a significant difference in AEC of 0.05. Also apparent in Table 7 is the excellent reproducibility in sample size requirements between experiments. Analyses of variance coupled with Tukey's test for grouping of means indicated that the AEC for the treatment BRH/REF was significantly different from all other treatments within a test for each test (Table 8). The fact that Nephtys from the treatment BRH/REF in both tests I and II had an AEC value of 0.92, which was significantly higher than AEC values for other treatments within a test, strongly suggests that these differences are real. This extremely high charge

would indicate that a highly oxidative and metabolically active state existed in these individuals. Nephtys were in a slightly less active metabolic state from other treatments, although AEC values obtained for all treatments in both tests I (2 Sept 83) and II (20 Sept 83) are indicative of healthy individuals. No other significant difference occurred among the other treatments in either test I or II (Table 8). These data lend credence to the fact that reproducibility within a test is exceptionally good. In order to determine the reproducibility between tests, analysis of variance coupled with Tukey's test was performed by comparison of the same treatment between tests for all four treatments. It is evident in Table 8 that the AEC for treatment REF/BRH differed significantly between tests. No other significant differences occurred among the other treatments between tests (Table 8). Although a significant difference occurred with the treatment REF/BRH between tests, the data support the fact that the reproducibility of AEC for the same treatment between tests is excellent (Table 8).

43. The data in Appendix A indicate that the AEC test is sensitive to changes in handling and acclimation procedures.

Table 8

The Response of Adenine Nucleotides in N. incisa After Treatment with BRH Sediment for 10 Days Under Laboratory Conditions (Tests I and II)

| Treatment Suspended/ Solid | n | µmol/g Wet Weight Tissue ATP | ADP | AMP | AEC | |
|---|---|---|---|---|---|---|
| | | Test I 02 Sept 83 | | | | |
| REF/REF | 10 | 1.36(0.04)* | 0.35(0.02)* | 0.06(0.005)* | 0.87(0.01)* | A** |
| BRH/REF | 10 | 1.33(0.09) | 0.19(0.02) | 0.02(0.001) | 0.92(0.01) | B |
| REF/BRH | 10 | 1.37(0.04) | 0.35(0.01) | 0.07(0.006) | 0.86(0.01) | A a† |
| BRH/BRH | 10 | 1.36(0.10) | 0.42(0.02) | 0.02(0.009) | 0.87(0.01) | A |
| | | Test II 20 Sept 83 | | | | |
| REF/REF | 10 | 1.42(0.06)* | 0.31(0.01)* | 0.04(0.01)* | 0.88(0.01)* | A** |
| BRH/REF | 9 | 1.30(0.16) | 0.20(0.04) | 0.03(0.005) | 0.92(0.01) | B |
| REF/BRH | 11 | 1.17(0.07) | 0.24(0.01) | 0.03(0.003) | 0.89(0.01) | A b† |
| BRH/BRH | 12 | 1.37(0.05) | 0.34(0.01) | 0.05(0.005) | 0.87(0.01) | A |

* Mean value of each sample with standard error of the mean in parentheses.

** Means with different letters are significantly different within a test at $\alpha = 0.05$.

† Means of similar treatments with different letters differ significantly between tests at $\alpha = 0.05$ and means with no letter are not significantly different.

## PART IV: DISCUSSION

44. Adductor muscle tissue of M. edulis and whole N. incisa were freeze clamped immediately after collection and stored in liquid nitrogen since it is important to inactivate the enzymes of the tissues very quickly to prevent degradation of ATP (Holm-Hansen and Booth 1966; Patterson et al. 1970; Ivanovici 1980). In addition, the choice of extraction method to maximize the extraction of adenine nucleotides is also important for the determination of their in vivo concentrations and AEC (Lundin and Thore 1975; Karl et al. 1978; Larsson and Olsson 1979; Karl 1980; Mendelssohn and McKee 1981). When working with N. incisa it is also important to anesthetize them to maximize the ATP concentration and AEC by eliminating all swimming activity once removed from the sediment prior to freeze clamping. Pamatmat (1982) reported that the polychaete Neanthes virens also showed unpredictable alternating periods of hyperactivity and rest when deprived of sediment. Skjoldal and Bamstedt (1977) reported that zooplankton underwent metabolic stress during capturing evidenced by a marked lowering of the ATP concentration and AEC.

45. We have had success in using PCA 6 percent (v/v) to extract adenine nucleotides from M. edulis as exemplified by high concentrations of ATP (> 2.80 μmol/g wet wt) with high AEC values (0.88) which were obtained consistently with untreated laboratory-held M. edulis (Zaroogian et al. 1982). However, this extraction procedure did not appear to be as suitable for extraction of adenine nucleotides from N. incisa since low concentations of ATP (0.52-0.57 μmol/g wet wt) with low AEC

values (0.73 - 0.74) were consistently obtained with both field-collected and laboratory-held worms. Initially, the extraction of adenine nucleotides, particularly ATP, was thought to be incomplete. However, during extraction trials with various concentations of PCA and TCA, loss of ATP with a corresponding decrease in AEC occurred in neutralized N. incisa homogenates which were stored for 1 week at -20°C. This indicated that ATPases were not being inactivated during the extraction process with either PCA or TCA at concentrations as high as 20 percent (v/v). Skjoldal and Bamstedt (1977) reported that 96 percent of the ATP in frozen zooplankton stored at -26°C degraded to AMP in 8 days. Wijsman (1976) found that when M. edulis tissues were homogenized in PCA, only part of the ATP was recovered and that recovery was dependent upon the time between homogenization and assay. He also determined that the ATP was not hydrolyzed by PCA itself. We did not see any decrease in ATP or AEC upon storage of M. edulis neutralized, PCA-extracted adductor muscle tissue for as long as 4 weeks at -20°C in this or in a previous study (Zaroogian et al. 1982). Ivanovici (1980) reported that ATP was stable for 4 weeks in neutralized PCA extracts of an estuarine mollusc (Pyrazus ebininus) when stored at -30°C. Wijsman (1976) used the total soft parts of M. edulis in his study, whereas in this study we used adductor muscle tissue and Ivanovici (1980) used columnar muscle tissue. The ATP degrading enzymes that Wijsman (1976) reported to be resistant to PCA inactivation may be found in tissues other than adductor muscle. Such remaining ATP degrading enzymes (ATPases) in PCA extracts have also been reported when PCA was used with microorganisms (Davison and Fynn 1974; Lundin and Thore 1975; Swedes et al. 1975).

46. Wijsman (1976) found TCA to be a better extractant of adenine nucleotides than PCA from M. edulis with no accompanying loss of ATP. In contrast, Wadley et al. (1980) found PCA the superior of four tested methods (PCA, TCA, $H_2SO_4$, and boiling bicarbonate buffer) when used with one gastropod and two bivalve species, whereas TCA yielded low AEC values. Trichloroacetic acid has been used to extract adenine nucleotides from microbial cell suspensions (Lundin and Thore 1975; Larsson and Olsson 1979) and has also been found to yield high adenine nucleotide concentrations with marine zooplankton (Ikeda and Skjoldal 1980; Skjoldal 1981) and M. edulis (Skjoldal and Barkati 1982). Our study corroborates that of Wadley et al. (1980) in that PCA extracted more adenine nucleotides than TCA in equimolar concentrations. However, higher concentrations of TCA (10 and 20 percent v/v) did yield concentrations of adenine nucleotides equal to those obtained with 6 percent PCA (v/v).

47. Adenine nucleotide extraction procedures which used heat were not considered for use intentionally since Karl and La Rock (1975) reported thermal gradients were likely to be established in fluid-solid mixtures due to variations in the kinetics of heat flow. Therefore, rapid enzyme inactivation and complete extraction of ATP would not occur.

48. Lundin and Thore (1975) reported that the enzymes responsible for the loss of ATP could be irreversibly inactivated by EDTA in combination with PCA, TCA, or heat and suggested that EDTA acts by destabilizing the enzymes by complexing metal co-factors. Methods incorporating EDTA for extraction of adenine nucleotides have been used with plants (Guinn and Eidenbock 1972; Mendelssohn and McKee 1981), bacteria (Chappelle and Levin 1968; Klofat et al. 1969; Lundin and Thore 1975; Thore et al. 1975),

zooplankton (Skjoldal 1981), mussels (Skjoldal and Barkati 1982), and polychaetes (Karl et al. 1978). In each case, highest concentrations of ATP were reported for the respective tissues analyzed. We also found this to be true in this study since the highest concentrations of adenine nucleotides were extracted when EDTA was included. The data, however, do not indicate that the ATP degrading enzymes are inactivated by the EDTA as suggested by Lundin and Thore (1975) since no differences in ADP and AMP concentrations occurred between the same tissue extracted with PCA or PCA containing EDTA. If, in fact, ATP concentrations were due to degradation of ATP, then an equivalent increase in ADP and/or AMP should occur if no AMP degrading enzymes are present. Thus it would appear that EDTA is facilitating the extraction of adenine nucleotides. Since additional work is required to elucidate the effect of EDTA in the extraction and stabilization of adenine nucleotides from N. incisa, the possibility of EDTA inactivating ATPases cannot be disregarded. The fact that ATP was lost in our neutralized extracts of N. incisa when stored at -20°C lends credence to the ATPase tenet and the effect of EDTA during storage remains to be tested.

49. A low energy charge most likely indicates a poor extraction of adenine nucleotides in tissue samples from individuals collected from a non-limiting environment. Thus, the intent here was not to quantify the adenine nucleotide concentrations but to develop an extraction procedure for N. incisa that consistently produces AEC ratios at levels representative of the in vivo levels previously reported for actively metabolizing cells in a non-limiting environment (Chapman et al. 1971). Uniformity within and among the extraction procedure is extremely important,

as this will minimize the variability associated with nucleotide extraction among treatments and tests. This in turn would be reflected in greater accuracy and reproducibility. Literature concerning AEC and polychaete worms is scant. However, Karl et al. (1978) determined the AEC ratios in the polychaete worm (Euzonus mucronata) and reported a value of 0.80. They used sulfuric acid as an extract and extracted tissue homogenates once in contrast to our twice. Schottler (1979) reported AEC values for three species of polychaete worms (Nereis) to be 0.88 to 0.90 for control worms under nonlimiting environmental conditions. Although he used dry weights, his concentrations of adenine nucleotides (ATP, 1.29 μmol/g; ADP, 0.32 μmol/g; AMP, 0.04 μmol/g) were comparable to ours when using a wet-to-dry ratio of 7:1 to calculate concentrations on a wet weight basis. He also extracted the tissue homogenates twice with PCA.

50. The AEC values for N. incisa from both tests I and II were indicative of actively metabolizing cells in a nonlimiting environment. Small differences in AEC (0.03 in this study) between treatments and tests that are significantly different can be detected with this method because of the sensitivity and low variability associated with the AEC values. However, although these small differences are statistically significant, the biological implications are not always meaningful. Thus, the statistically significant difference between tests for the REF/BRH treatments does not necessarily infer a difference in health condition.

51. During the tests, the worms appeared to burrow preferentially in the REF sediment regardless of treatment or position of the REF sediment in relation to the BRH sediment.

52. When BRH sediment was suspended over the REF sediment the worms were actively burrowing and feeding, thus they were metabolically active and the highest AEC values were obtained with this treatment. It would suggest that BRH is somewhat higher in nutritive value than the REF sediment.

53. The AEC values reported for M. edulis taken from a non-limiting environment are: 0.91 (Wijsman 1976), 0.90 (Skjoldal and Barkati 1982), and 0.85 to 0.88 (Zaroogian et al. 1982).

54. Mussels from all treatments in this study had lower AEC values than those obtained at the start of treatment. However, the AEC values obtained with mussels from the 100 percent REF and 100 percent BRH treatments in test I (17 Nov 83) and with mussels from all treatments in test II (19 Mar 84) were representative of actively metabolizing cells in a nonlimiting environment (Chapman et al. 1971). Although the AEC values in these treatments indicated a healthy condition, the values were at the lower end of the spectrum for this health condition. However, that the mussels treated with REF sediment had lower AEC values than those at the start of treatment could indicate the following:

a. Holding conditions are not entirely adequate.

b. Food quality is poor.

c. Food supply is inadequate.

d. Because of the particulate load (sediment plus food) the mussels are expending more energy (faster filtration rate) in relation to the energy return from the amount of food assimilated.

55. The fact that a greater decrease was observed in the AEC value after 26 days of treatment with 50 percent BRH and 50 percent REF sediment than with 100 percent BRH sediment in test I (17 Nov 83) and test II (19 Mar 84) at first appeared to be a paradox, but upon further examination seemed consistent with observations reported by others (Davenport and Manley 1978; Giesy et al. 1983). Mytilus edulis is able to undergo anaerobic respiration for long periods of time (5 to 7 days) (Wijsman 1976). Shell closure and anaerobiosis may enable M. edulis to avoid continuous ingestion of toxicants which they are able to sense. The lower BRH sediment concentration used in this study may have contained contaminants whose concentrations were below the threshold concentration at which the mussels closed to avoid exposure. To suggest or imply that the mussels treated with 100 percent BRH closed their shells and survived anaerobically for 26 days would be inane. However, it is feasible and possible that these mussels opened their shells intermittently for feeding and excretion. This is supported by the observation during test I (17 Nov 83) and test II (19 Mar 84) that mussels were filtering less in the 100 percent BRH treatment than in the other two treatments.*

56. The reproducibility between tests was excellent with identical AEC values for the same treatment between tests except for the 50 percent REF/50 percent BRH treatment which differed significantly. This difference is an accurate reflection of the metabolic state of the mussels since it is obvious in Table 1 that mussels in the 50 percent REF/50 percent BRH treatment in test I (17 Nov 83) experienced higher concentrations of

---

* Personal communication, Dianne Black, March 1984, U.S. Environmental Protection Agency.

suspended particulate than their counterparts in test II (19 Mar 84). In addition, 13 percent mortality was recorded for the 50 percent REF/50 percent BRH treatment during test I (17 Nov 83) as opposed to no mortality for the same treatment during test II (19 Mar 84) (Table 1). These facts also helped to explain the lower AEC value (0.75) obtained with mussels from the 50 percent REF/50 percent BRH treatment in test I (17 Nov 83) when compared to the AEC value (0.80) obtained with mussels from the same treatment in test II (19 Mar 84). Although a casual interpretation of the data would suggest that improvement in reproducibility of AEC measurement for the 50 percent REF/50 percent BRH treatment is wanting, an in depth study of the data indicated otherwise, in that the AEC accurately reflected the variation in conditions that existed within and between the two tests (Table 1). Thus it would appear that the metabolic state and health condition of the mussels in the 50 percent REF/50 percent BRH treatment from test II (19 Mar 84) are better than their counterparts in test I (17 Nov 83) as indicated by the AEC.

## PART V: CONCLUSIONS

57. The AEC appears to be a highly sensitive method for measuring stress in N. incisa and M. edulis treated with dredged material under laboratory conditions. The low variability associated with AEC measurements and the excellent reproducibility between tests would support this conclusion.

58. Adenylate energy charge is an excellent representation of the actual metabolic state and health condition of N. incisa and M. edulis.

59. Both N. incisa and M. edulis are excellent species with which AEC can be used to accurately assess their metabolic state and health condition when exposed to sublethal environmental perturbations.

60. This investigation is the first phase in developing field-verified bioassessment evaluations for the Corps of Engineers and the US Environmental Protection Agency regulatory program for dredged material disposal. This report is not intended for regulatory purposes; appropriate assessment methodologies that are field verified will be available at the conclusion of this program.

## REFERENCES


Adam, H. 1963. "Adenosine-5'-phosphate: Determination with phosphoglycerate kinase," Methods of Enzymatic Analysis (Edited by Bergmeyer, H.V.) p 541. Verlag Chemie Weinheim, Academic Press, New York.

American Society for Testing and Materials. 1980. "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians," ASTM E-729-80, Philadelphia, PA.

Atkinson, D.E. 1971. "Regulation of Enzyme Function," Annual Review of Microbiology, Vol 23, pp 47-68.

Atkinson, D.E., and Walton, G.M. 1967. "Adenosine Triphosphate Conservation in Metabolic Regulation; Rat Liver Citrate Cleavage enzyme," Journal of Biological Chemistry, Vol 242, pp 3239-3241.

Ball, W.J., and Atkinson, D.E. 1975. "Adenylate energy charge in Saccharomyces cerevisiae during starvation," Journal of Bacteriology, Vol 121, pp 975-982.

Behm, C.A., and Bryant, C. 1975. "Studies on regulatory metabolism in Moniezia expansa: The role of phosphofructo kinase (with a note on pyruvate kinase)," International Journal of Parasitology, Vol 5, pp 339-346.

Bergmeyer, H.V. 1965. "Cell and tissue disintegration," Methods of Enzymatic Analysis. Edited by H.V. Bergmeyer. p 1064. Academic Press, New York.

Chapman, A.G., and Atkinson, D.E. 1977. "Adenine nucleotide concentrations and turnover rates: their correlation with biological activity in bacteria and yeast," Advances in Microbial Physiology, Vol 15, pp 253-306.

Chapman, A.G., Fall, L., and Atkinson, D.E. 1971. "Adenylate Energy Charge in Escherichia coli During Growth and Starvation," Journal of Bacteriology, Vol 108, pp 1072-1086.

Chappelle, E.W., and Levin, G.V. 1968. "Use of the Firefly Bioluminescent Reaction for Rapid Detection and Counting of Bacteria," Biochemical Medicine, Vol 2, pp 41-52.

Christensen, J.P., and Devol, A.H. 1980. "Adenosine Triphosphate and Adenylate Energy Charge in Marine Sediments," Marine Biology, Vol 56, pp 175-182.

Davenport, J., and Manley, A. 1978. "The Detection of Heightened Seawater Copper Concentrations by the Mussel Mytilus edulis," Journal Marine Biological Association United Kingdom, Vol 58, pp 843-850.

Davison, J.A., and Fynn, G.H. 1974. "The Assay of ATP by the Luciferin-luciferese Method. Interference by a Bacterial Phosphatase Enzyme Stable to Perchlorate Treatment," Analytical Biochemistry, 58, pp 632-637.

Dean, D., and Mazurkiewicz, M. 1975. "Methods of Culturing Polychaetes," Culture of Marine Invertebrate Animals. Eds. Smith, W.L. and Chanley, M.H. Plenum Press, New York.

Dickson, G.W., Giesy, J.P., and Briese, L.A. 1982. "The Effect of Chronic Cadmium Exposure on Phosphoadenylate Concentrations and Adenylate Energy Charge of Gills and Dorsal Muscle Tissue of Crayfish," Environmental Toxicolology and Chemistry, Vol 1, pp 147-156.

Giesy, J.P., Denzer, S.R., Duke, C.S., and Dickson, G.W. 1981. "Phospho-adenylate Concentrations and Energy Charge in Two Freshwater Crustaceans: Responses to Physical and Chemical Stressors," Versuchungen Internationale Vereinigung Limnologie, Vol 21, pp 205-220.

Giesy, J.P., Duke, C.S., Bingham, R.D. and Dickson, G.W. 1983. "Changes in Phosphoadenylate Concentrations and Adenylate Energy Charges as an Integrated Biochemical Measure of Stress in Invertebrates: The Effects of Cadmium on the Freshwater Clam Corbicula fluminea," Toxicology and Environmental Chemistry, Vol 6, pp 259-295.

Guillard, R.R.L. 1975. "Culture of Phytoplankton for Feeding Marine Invertebrates," Culture of Marine Invertebrate Animals, Eds. Smith, W.L., and Chanley, M.H. Plenum Publishing Corporation, New York.

Guinn, G., and Eidenbock, M.P. 1972. "Extraction, Purification, and Estimation of ATP from Leaves, Floral Buds and Immature Fruits of Cotton," Analytical Biochemistry, Vol 50, pp 89-97.

Holm-Hansen, O., and Booth, E.R. 1966. "The Measurement of Adenosine Triphosphate in the Ocean and Its Ecological Significance," Limnology and Oceanography, Vol 11, pp 510-519.

Hoya, F., Waiwood, B.A., and Johnston, D.W. 1983. "Adenylate Energy Charge and ATPase Activity of Lobster (Homarus americanus) During Sublethal Exposure to Zinc," Aquatic Toxicology, Vol 3, pp 115-126.

Ikeda, J., and Skjoldal, H.R. 1980. "The Effect of Laboratory Measurements to the Ecology of Marine Zooplankton. IV. Changes in Physiological Activites and Biochemical Components of Acetes sibogae australis and Acartia australis after Capture," Marine Biology, Vol 58, pp 285-293.

Ivanovici, A.M. 1980. "Adenylate Energy Charge in Pyrazus ebeninus in Response to Salinity," Comparative Biochemistry and Physiology, Vol 66A, pp 43-57.

Karl, D.M. 1980. "Cellular Nucleotide Measurements and Applications in Microbial Ecology," Microbiology Reviews, pp 739-796.

Karl, D.M., Haugness, J.A., Campbell, L., and Holm-Hansen, O. 1978. "Adenine Nucleotide Extraction from Multi-cellular Organisms and Beach Sand: ATP Recovery, Energy Charge Ratios and Determination of Carbon/ATP Ratios," Journal of Experimental Marine Ecology, Vol 34, pp 163-181.

Karl, D.M., and Holm-Hansen, O. 1978. "Methodology and Measurement of Adenylate Energy Charge Ratios in Environmental Samples," Marine Biology, Vol 48, pp 185-197.

Karl, D.M., and La Rock, P.A. 1975. "Adenosine Triphosphate Measurements in Soil and Marine Sediments," Journal of Fisheries Research Board, Canada, Vol 32, pp 599-607.

Klofat, W., Picciolo, G., Chappelle, E.W., and Freese, E. 1969. "Production of adenosine triphosphate in normal cells and sporulation mutants of Bacilus subtilis," Journal of Biological Chemistry, Vol 244, pp 3270-3276.

Lake, J., Hoffman, G., and Schimmel, S. 1984. "Bioaccumulation of Contaminants from Black Rock Harbor Dredged Material by Mussels and Polychaetes," Technical Report D-84- , prepared by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, R.I., for the U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

Lamprecht, W., and Trautschold, I. 1974. "Adenosine-5'-triphosphate: Determination with Hexokinase and Glucose-6-phosphate Dehydrogenase," Methods of Enzymatic Analysis, Vol 4 (Edited by Bergmeyer, H.V.), pp 2101-2110, Second English edition, Verlag Chemie Weinheim, Academic Press, New York.

Larsson, C.M., and Olsson, T. 1979. "Firefly Assay of Adenine Nucleotides From Algae: Comparison of Extraction Methods," Plant and Cell Physiology, Tokyo, Vol 20, pp 145-155.

Lundin, A., and Thore, A. 1975. "Comparison of Methods for Extraction of Bacterial Adenine Nucleotides Determined by Firefly Assay," Applied Microbiology, Vol 30, pp 713-721.

Mendelssohn, I.A., and McKee, K.L. 1981. "Determination of Adenine Nucleotide Levels and Adenylate Energy Charge Ratio in Two Spartina species," Aquatic Botany, Vol 11, pp 37-55.

Montague, M.D., and Dawes, E.A. 1974. "The Survival of Peptococcus prevotii in Relation to the Adenylate Energy Charge," Journal of General Microbiology, Vol 80, pp 291-299.

Pamatmat, M.M. 1982. "Metabolism of Burrowing Polychaete: Precaution Needed When Measuring Toxic Effects," Marine Pollution Bulletin, Vol 13, pp 364-367.

Patterson, J.W., Brezonik, P.L., and Putnam, H.D. 1970. "Measurement and significance of adenosine triphosphate in activated sludge," Environmental Science Technology, Vol 4, pp 569-575.

Pesch, C.E. and Hoffman, G.L. 1983. "Interlaboratory Comparison of a 28-day Toxicity Test with the Polychaete Neanthes arenaceodentata," Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802. W.E. Bishop, R.D. Cardwell, and B.B. Heidolph, eds. American Society for Testing and Materials, Philadelphia, PA, pp 482-493.

Rainer, S.F., Ivanovici, A.M., and Wadley, V.A. 1979. "The Effect of Reduced Salinity on Adenylate Energy Charge in Three Estuarine Molluscs," Marine Biology, Vol 54, pp 91-99.

Ridge, J.W. 1972. "Hypoxia and the Energy Charge of the Cerebral Adenylate Pool," Biochemical Journal, Vol 127, pp 351-355.

Romano, J.C., and Daumas, R. 1981. "Adenosine Nucleotide "Energy Charge" Ratios as an Ecoyphysiological Index for Microplankton Communities," Marine Biology, Vol 62, pp 281-296.

Schottler, V. 1979. "On the Anaerobic Metabolism of Three Species of Nereis (Annelida)," Marine Ecology Progress Series, Vol 1, pp 249-254.

Skjoldal, H.R. 1981. "ATP Concentration and Adenylate Energy Charge of Tropical Zooplankton from Waters Inside the Great Barrier Reef," Marine Biology, Vol 62, pp 119-123.

Skjoldal, H.R., and Bakke, T. 1978. "Relationship Between ATP and Energy Charge During Lethal Metabolic Stress of the Marine Isopod Cirolana borealis," Journal of Biological Chemistry, Vol 253, pp 3355-3356.

Skjoldal, H.R., and Bamstedt, V. 1977. "Ecobiochemical Studies on the Deep-water Pelagic Community of Korsfjorden, Western Norway. Adenine nucleotides in zooplankton," Marine Biology, Vol 42, pp 197-211.

Skjoldal, H.R., and Barkati, S. 1982. "ATP Content and Adenylate Energy Charge of the Mussel Mytilus edulis During the Annual Reproductive Cycle in Lindaspollene, West Norway," Marine Biology, Vol 70, pp 1-6.

Snedecor, G.W., and Cochran, W.G. 1980. Statistical Methods, Iowa State University Press, Ames, Iowa.

Swedes, J.S., Sedo, R.J., and Atkinson, D.E. 1975. "Relation to Growth and Protein Synthesis to the Adenylate Energy Charge in an Adenine Requiring Mutant of Escherichia coli," Journal of Biological Chemistry, Vol 250, pp 6930-6940.

Thore, A., Ansehn, S. Lundin, A., and Bergman, S. 1975. "Detection of Bacteriuria by Luciferase Assay of Adenosine Triphosphate," Journal of Clinical Bacteriology, Vol 1, pp 1-8.

Vetter, R.D., and Hodson, R.E. 1982. "Use of Adenylate Concentrations and Adenylate Energy Charge as Indicators of Hypoxic Stress in Estuarine Fish," Canadian Journal of Fisheries and Aquatic Science, Vol 39, pp 535-541.

Vetter, R.D., Hodson, R.E., and Arnold, C. 1983. "Energy Metabolism in a Rapidly Developing Marine Fish Egg, the Red Drum (Sciaenaps ocellata)," Canadian Journal of Fisheries and Aquatic Science, Vol 40, pp 627-634.

Wadley, V.A., Ivanovici, A.M., and Rainer, S.F. 1980. "A Comparison of Techniques for the Measurement of Adenine Nucleotides in Three Species of Estuarine Mollusc," Report Division Fisheries Oceanography, C.S.I.R.O., Vol 129, pp 1-23.

Wiebe, W.J., and Bancroft, K. 1975. "Use of the Adenylate Energy Charge Ratio to Measure Growth State of Natural Microbial Communities," Proceedings National Academy Sciences, Vol 72, pp 2112-2115.

Wijsman, T.S.M. 1976. "Adenosine Phosphates and Energy Charge in Different Tissues of Mytilus edulis Under Aerobic and Anaerobic Conditions," Journal of Comparative Physiology, Vol 107, pp 129-140.

Zaroogian, G.E., Gentile, J.H., Heltshe, J.F., Johnson, M., and Ivanovici, A.M. 1982. "Application of Adenine Nucleotide Measurements for the Evaluation of Stress in Mytilus edulis and Crassostrea virginica," Comparative Biochemistry and Physiology, Vol 71B, pp 643-649.

## APPENDIX A: EFFECTS OF HANDLING AND ACCLIMATION PROCEDURES ON AEC

The data in Table A1 indicate that the AEC test is sensitive to changes in handling and acclimation procedures. Although the charges in the 2 September 83, 20 September 83, and the 10 November 83 tests indicate healthy individuals, the results of the 10 November 83 test were different from the results of the 2 September 83 and 20 September 83 tests which were essentially identical. Therefore, strict adherence to uniformity in handling and acclimation of N. incisa is imperative since the AEC measurement is sensitive enough to detect differences due to these parameters. This sensitivity of AEC to alterations in test procedures would also be reflected in the reproducibility of tests. Analysis of variance and Tukey's test for pairwise comparisons indicated that AEC values for each treatment in the 10 November 83 test were significantly different from the respective treatments in both the 2 September and 20 September 83 tests.

Table A1

The Response of Adenine Nucleotides in N. incisa

After Treatment with BRH Sediment for 10 Days

under Laboratory Conditions (Test III)

| Treatment suspended/ solid | n | µMol/g Wet Weight Tissue ATP | ADP | AMP | AEC | |
|---|---|---|---|---|---|---|
| | | Test III 10 Nov 83 | | | | |
| REF/REF | 10 | 1.63(0.33)* | 0.59(0.05)* | 0.06(0.01)* | 0.82(0.01)* | A** |
| BRH/REF | 10 | 2.11(0.38) | 0.62(0.05) | 0.11(0.01) | 0.83(0.01) | A |
| REF/BRH | 10 | 1.68(0.27) | 0.60(0.03) | 0.08(0.01) | 0.82(0.01) | A |
| BRH/BRH | 10 | 1.66(0.33) | 0.54(0.04) | 0.11(0.01) | 0.82(0.01) | A |

* Mean value of each sample with standard error of mean in parentheses.

** Means with the same letter are not significantly different at $\alpha = 0.05$.

END

FILMED

9-85

DTIC